PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 33/543 A1	(11) International Publication Number: WO 94/18564
	(43) International Publication Date: 18 August 1994 (18.08.94)

FILE COPY

(21) International Application Number: PCT/FI94/00047

(22) International Filing Date: 1 February 1994 (01.02.94)

(30) Priority Data:
930440 1 February 1993 (01.02.93) FI

(71) Applicant *(for all designated States except US)*: LABSYSTEMS OY [FI/FI]; PL 8, FIN-00881 Helsinki (FI).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: TUUNANEN, Jukka [FI/FI]; Koivikkotie 20 C, FIN-00630 Helsinki (FI).

(74) Agent: RUSKA & CO OY; Runeberginkatu 5, FIN-00100 Helsinki (FI).

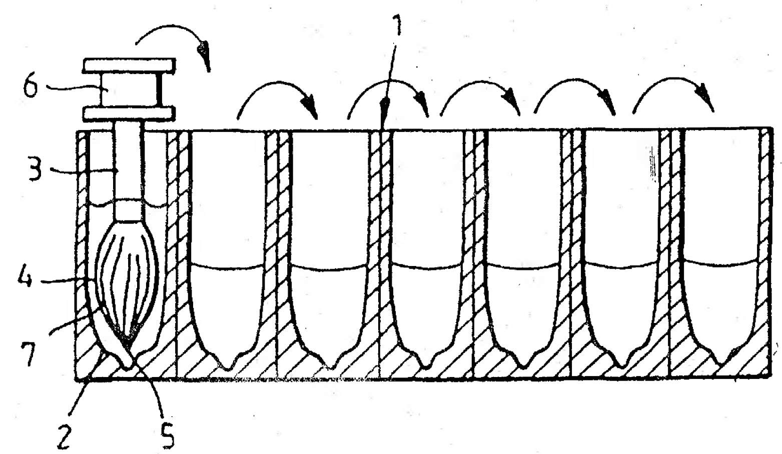
(81) Designated States: FI, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
In English translation (filed in Finnish).

(54) Title: SOLID PHASE IMMUNOASSAY WITH CARRIERS MATCHING THE SHAPE OF SAMPLE WELLS

(57) Abstract

The invention concerns a solid-phase determination method and equipment and an adapter for use in these. In the method a sample is allowed to react with a separating reagent bound to the outer surface of a separate solid-phase body (4), whereafter the body is removed from the vessel and is taken to a measuring vessel, if required through one or several intermediate step vessels. At least one vessel contains a medium needed in a determination step to be performed therein when the phase body is brought into this vessel. The invention is especially suitable for use in automatic immunodetermination systems.



Solid phase immunoassay with carriers matching the shape of sample wells

FIELD OF THE INVENTION

The invention concerns a solid-phase determination method and equipment as well as an adapter for use in these. The invention is especially suitable for use in automatic immunodetermination systems.

BACKGROUND OF THE INVENTION

Solid-phase immunodetermination is usually carried out in one vessel so that the analyte to be determined in the sample is first allowed to react with a separating reagent bound in a solid phase, whereupon the other determination steps are carried out in the same vessel. The troublesome thing here is that you must do much dosing and removing of fluids. When there are several different determinations, a large stock of different reagents is also required.

Specification EP-A-194789 also presents a system, wherein the determination is carried out by using several vessels. The solid phase is formed by a thin strip which is moved from one vessel to another. The vessels contain the reagents needed in the determination method.

DESCRIPTION OF THE INVENTION

A determination method as defined in claim 1 has now been invented. Advantageous applications of the invention are defined in the following claims.

As used herein, a separating reagent generally means such a substance which will react with the analyte to be determined and will bind it in the solid phase. In immunodeterminations the separating reagent is usually an antigen or antibody. As used herein, a medium generally means a solution to be used at some stage of the determination, such as a reaction liquid or a washing fluid.

The solid phase used in the method is the outer surface of a solid body separate from the reaction vessel, and the determination steps are performed in two or more vessels. The solid-phase body is kept in the vessel containing the sample

either directly detectable or of such a type which will release some detectable compound, especially from a substrate. Detection usually takes place fluorometrically, luminometrically, absorptiometrically or radiometrically.

There is no risk of contamination in the method, because the sample is not drawn into the equipment from the plate vessels. Besides, the method can be implemented by using simple and very reliably-operating automatic equipment. In addition, the phase body works as an effective agitating piston in the reaction vessel.

The invention is suitable, for example, for immunological, DNA-hybridization or hormone determinations.

The solid body is preferably in one piece. However, it may also be assembled into a separate frame from several parts, for example, rings.

To speed up mass transport and thus the necessary reaction time, the medium should be agitated during the reaction. This is preferably done by moving the phase body. It is especially advantageous to move the remover vertically, whereby the medium must flow through the gap between the remover and the vessel, thus blending very efficiently. To make mixing more effective, the body is constructed so wide that a gap of a suitable narrowness is formed between the vessel and the remover. Mixing can also be promoted by suitable designing of the body and the vessel.

The vessel unit forms a plate for use in one determination. The phase body may be packed into some vessel in the plate. The vessels to be used in the different steps may also be of different sizes.

The vessels are preferably closed with a film, which is punctured for carrying out the method. The film may be punctured by using the phase body, but using a separate puncturing point is recommended. The point may have cutting blades forming strips which will tear in a controlled manner. In the equipment, the puncturing point may be attached to the same actuator as the phase body. The top edge of the vessel is preferably provided with an extension against which the strips of the punctured film can rest. Closed vessels may have an inert gas phase to improve durability. The plate

The body 4 is oval and has a point 5 at its bottom end. The vessel 2 bottom is shaped correspondingly. At its upper end rod 3 has a handle 6 which is suitable for robotics and at which the rod can be grasped for exact control of its horizont-al and vertical positions. The body surface has suitable protrusions and cavities 7 to increase the surface area. In this embodiment they are grooves leading toward the point.

The sample to be examined is brought first into the first well 2 in plate 1 containing a suitable diluter, if required, whereafter phase body 4 is immersed into it. The analyte possibly existing in the sample is now allowed to react with the separating reagent to form an immunocomplex. After this incubation the phase body is moved into the second well containing a first washing fluid, into the third well containing another washing fluid and into the fourth well containing an enzyme conjugate adhering to the immunocomplex. After tracer incubation the phase body is again moved by way of two washing wells for measurement in the last well containing a substrate for the enzyme, from which the enzyme will release a compound that can be detected fluorometrically. After the substrate reaction the phase body is taken aside and fluorometric measurement is performed in such a way that both exitation radiation and emission radiation are led through the well mouth.

During the incubations and washes the phase body is moved back and forth in a vessel, whereby the medium will blend effectively.

Plate 1 can be made of some cheap plastic material, because light need not be led through the well wall. For this reason, as simple a manufacturing technology as possible can also be used. To reduce background radiation the material is preferably opaque.

Luminometric determinations can be carried out in a similar way.

If the reaction result is measured absorptiometrically, the measuring vessel must be transparent or radiation must be provided through special arrangements (for example, using a reflecting bottom) from the measuring vessel to the detector.

out the react-ion result. Phase bodies and possible means for penetrating the film closures of the wells are placed on an arm 20. The equip-ment also includes a thermostatic heater for keeping the plates at the desired temperature.

A phase body is attached to arm 20 for each sample plate. Samples are dosed into the first well in the plates 1 in cassette 14 and the cassette is pushed in. It moves into its extreme position where identifying device 18 reads code 15, whereby a control unit receives the data needed for performing the determination. The phase bodies are lowered into the first wells. After incubation the phase bodies are taken up, the plate is moved one step forward and the second step is performed. The process goes on in this way from one well to another, and finally measuring is done in the last well. The determination result of each plate is shown on display 21.

All determinations may be different, provided that they can be performed with the number of wells available in the plate. All wells may not be needed in all determinations, in which case there is no medium in them.

Such equipment may of course also be used where both the detector end and the phase bodies are attached to the same arm.

Figure 4 shows equipment of a modular type where six cassettes can be handled at the same time.

In this embodiment the available plates 1 have a code 15 at the end of the first well. Cassettes 14 are preheated in incubator 22 and they are pushed into the equipment with the code end first through feeding opening 16. The phase bodies needed for each cassette are located on arms 20 at the places of the corresponding plates.

The equipment has a common transversely movable detector end 17 provided with an identifying device 18 and with a measuring device 19. The identifying device reads code 15 in each plate, whereupon the cassette moves inward to its extreme position, wherein the sample and possibly deluting agent, too, are metered into the first well. Dashed line 23 shows the path of movement of the dosing device. The cassette is then moved outwardly so that the first well is located

CLAIMS

1. Solid-phase determination method wherein the sample possibly containing an analyte to be determined is allowed to react in a medium contained in a reaction vessel with the analyte's separating reagent bound to the surface of a solid-phase body separate from the vessel into an analyte reagent complex, and one or several intermediate steps are performed, if required, whereafter any formed complex is established, **characterized in that**

- the sample is allowed to react with a separating reagent bound in a solid phase to the outer surface of a solid-phase body which has the cross-sectional form of a reaction vessel,
- after the reaction the phase body is removed from the vessel,
- the phase body is moved, if required through one or several intermediate step vessels performing intermediate steps in a medium in these, into a measuring vessel and
- a possibly formed complex is detected in the measuring vessel,

whereby at least one vessel contains a medium required in the determination step to be performed therein when the phase body is brought into this vessel.

2. Method as defined in claim 1, **characterized in that** a medium is agitated in a vessel during at least some determination step.

3. Method as defined in claim 2, **characterized in that** the medium is agitated by using the phase body, preferably by moving the phase body vertically.

4. Method as defined in anyone of claims 1 - 3, **characterized in that** in one or several determination steps, preferably in the first step, from which the phase body is moved to the following step, the sample or any formed complex is allowed to react with a substance bound in a solid phase and added to the vessel wall or to the medium and remaining in the vessel, which substance will bind any substances disturbing the following determination steps.

5. Equipment for determination of an analyte from a sample possibly containing it by allowing the sample to react in a medium contained in a vessel with the analyte's separating

12. A set of means for use in the determination of an analyte from a sample possibly containing it by allowing the sample to react in a medium contained in a vessel with the analyte's separating reagent bound in a solid phase separately from the vessel to form an analyte-separating-reagent-complex and, if required, after possible intermediate steps by detecting a possibly formed complex, **characterized in that** the set of means comprises

- a reaction vessel (2) and for this a solid-phase body (4) with a cross-sectional shape similar to the reaction vessel and with the separating reagent bound in a solid phase onto its outer surface,
- for intermediate steps possibly to be performed in a medium, one or several vessels or measuring vessels for detecting any possibly formed complex,

whereby at least one vessel contains a medium needed for an intermediate step to be performed in it.

13. Set of means as defined in claim 12, **characterized in that** the vessels contain all mediums required in the measurement.

14. Set of means as defined in claim 12 or 13, **characterized in that** at least one vessel, and preferably all vessels, are closed with a penetrable film.

15. Set of means as defined in claim 14, **characterized in that** at least some closed vessel contains an inert gas phase.

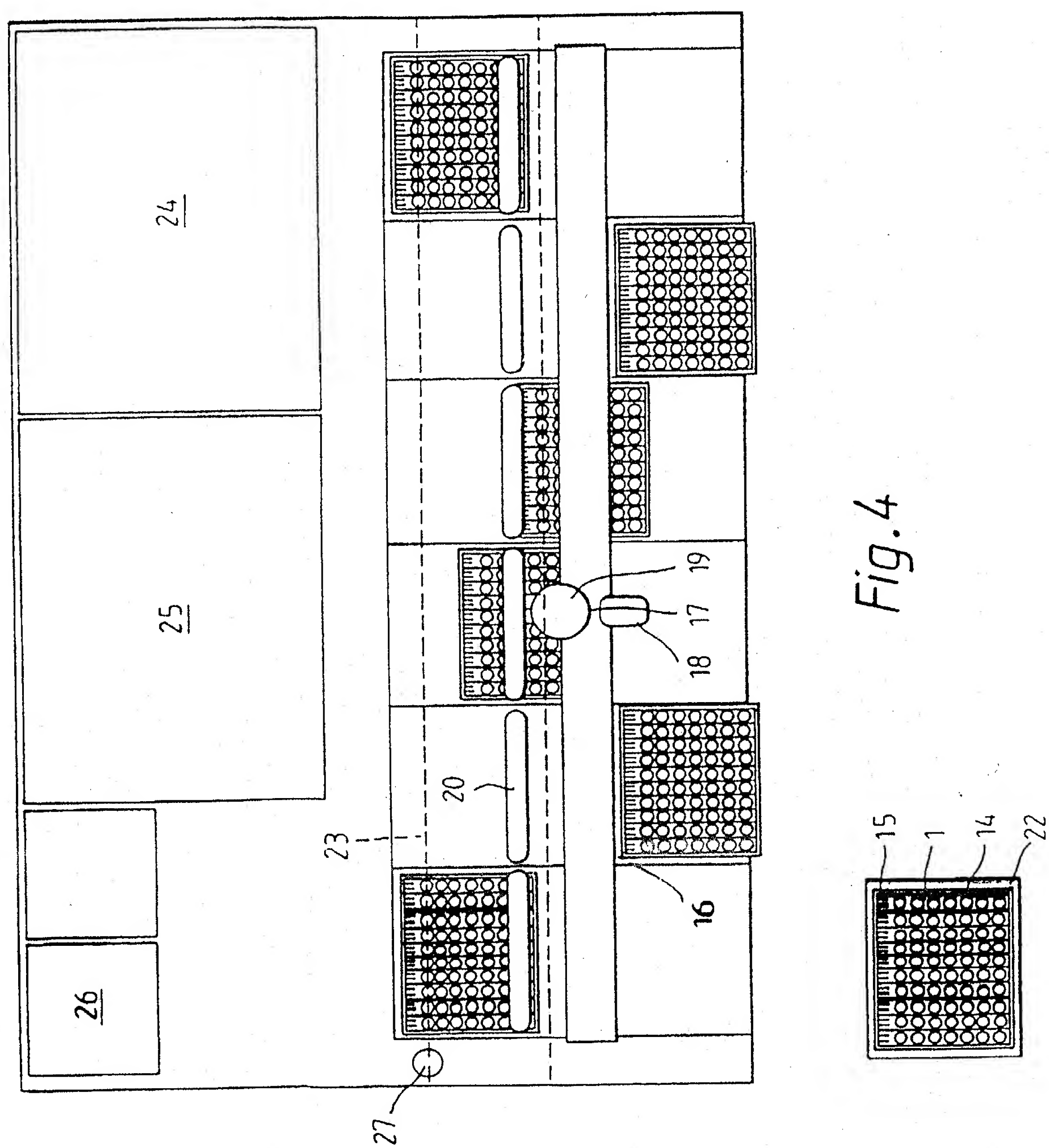


Fig. 4

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB, A, 1414479 (ABBOTT LABORATORIES), 19 November 1975 (19.11.75), see fig 1-4 and page 4, lines 20-28 and claims --	1-15
A	Patent Abstracts of Japan, Vol 12,No 203, P-715, abstract of JP, A, 63-5263 (YASUNOBU TSUKIOKA), 11 January 1988 (11.01.88) --	1-15
Y	DE, A1, 2824742 (R N PIASIO), 15 February 1979 (15.02.79), see eg. page 33, last paragraph - page 34, first paragraph and fig 5 --	6-8
Y	US, A, 4200613 (C P ALFREY), 29 April 1980 (29.04.80), column 7, line 40 - line 45 --	2,3,9,10
Y	EP, A1, 0027008 (VENTREX LABORATORIES INC.), 15 April 1981 (15.04.81) -- ---------	4

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP-A2-	0042755	30/12/81	SE-T3-	0042755	
			DE-A-	3176537	23/12/87
			DE-A-	3176854	29/09/88
			EP-A,B-	0111762	27/06/84
			SE-T3-	0111762	
			EP-A-	0044219	20/01/82
GB-A-	2147698	15/05/85	DE-A-	3242393	26/05/83
			FR-A,B-	2516654	20/05/83
			GB-A,B-	2114738	24/08/83
			JP-A-	58127143	28/07/83
GB-A-	1414479	19/11/75	AU-A-	5021572	20/06/74
			CA-A-	988419	04/05/76
			CH-A-	566126	15/09/75
			DE-A-	2262479	28/06/73
			FR-A-	2170506	14/09/73
			JP-A-	48071700	27/09/73
			US-A-	3826619	30/07/74
DE-A1-	2824742	15/02/79	AU-B-	521002	11/03/82
			AU-A-	3692778	13/12/79
			BE-A-	868016	02/10/78
			CA-A-	1109791	29/09/81
			FR-A,B-	2394088	05/01/79
			GB-A-	1584129	04/02/81
			JP-A-	54063796	22/05/79
			SE-B,C-	446666	29/09/86
			SE-A-	7806400	11/12/78
			US-A-	4197287	08/04/80
			US-A-	4225575	30/09/80
US-A-	4200613	29/04/80	DE-A,C-	2824164	15/03/79
			GB-A-	1585948	11/03/81
EP-A1-	0027008	15/04/81	AU-A-	6278480	02/07/81
			CA-A-	1149279	05/07/83
			JP-A-	56096248	04/08/81
			US-A-	4378344	29/03/83

A. CLASSIFICATION OF SUBJECT MATTER

IPC : G01N 33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC : G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP, A2, 0042755 (UNILEVER PLC), 30 December 1981 (30.12.81), page 7; page 8, line 15 - line 31; page 14, line 23 - line 35, claims	1-3,5,6-15
Y		4,7
	--	
X	GB, A, 2147698 (UNILEVER PLC), 15 May 1985 (15.05.85), see page 1, lines 32-80, page 2, left column and claims	1,2,5,6,8-15
Y		3,4,7
	--	
Y	Patent Abstracts of Japan, Vol 7,No 75, P-187, abstract of JP, A, 58-5657 (TOYO JOZO K.K.), 13 January 1983 (13.01.83)	7
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" erlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 May 1994	08 -05- 1994
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl Olof Gustafsson Telephone No. +46 8 782 25 00

Fig.1

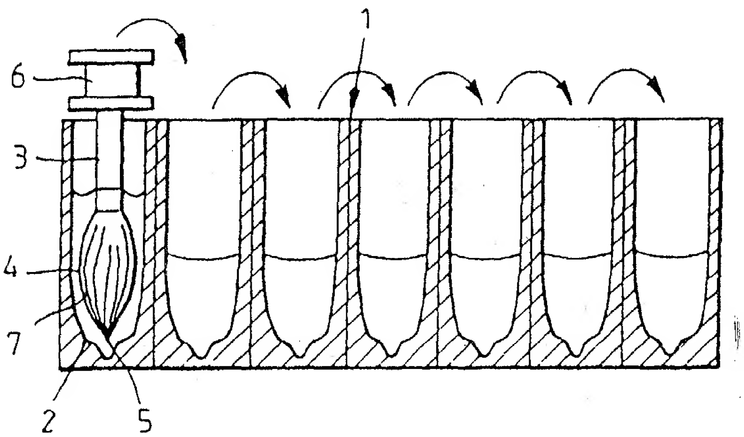


Fig.2

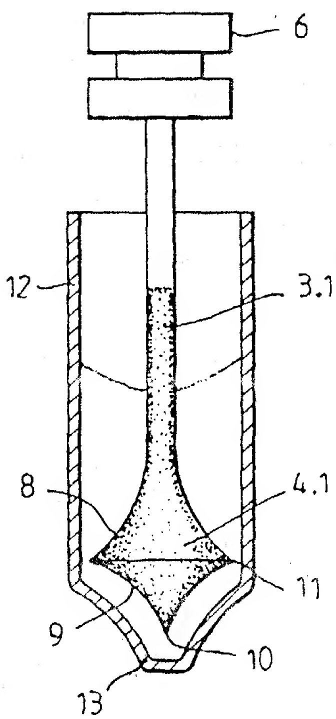
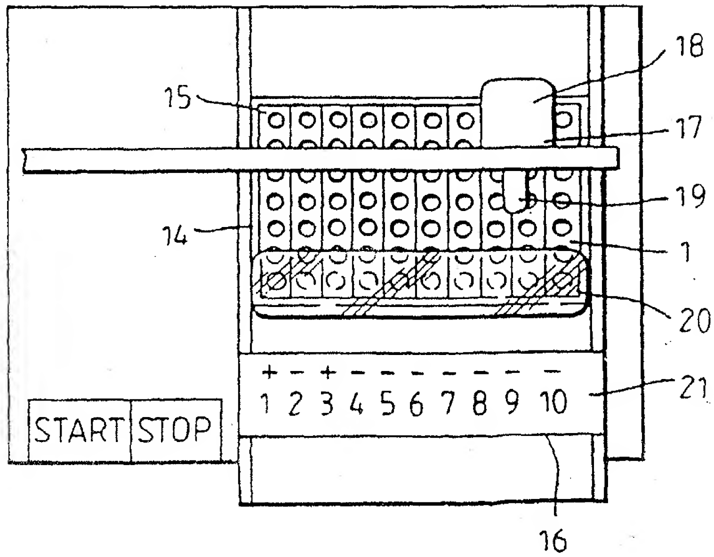


Fig.3



reagent bound in a solid phase separately from the vessel to form an analyte-separating-reagent-complex and, if required after possible intermediate steps, by establishing any formed complex, **characterized in that** the equipment comprises

- a reaction vessel (2) to be placed in the equipment and for the reaction vessel a solid-phase body (4) shaped with a cross-section similar to the reaction vessel and on whose outer surface the separating reagent is bound in a solid phase,

- a measuring vessel and a measuring device for detecting a possibly formed complex,

- one or several vessels for possible intermediate steps to be performed in a medium, and

- an actuator for removing the phase body from the vessel and for moving it into another vessel,

whereby at least one vessel contains a medium for use in a determination step to be performed in it.

6. Equipment as defined in claim 5, **characterized in that** the phase body has grooves (7) or protrusions to increase the surface area of the solid phase.

7. Equipment as defined in anyone of claims 5 - 6, **characterized in that** to promote flowing of fluid the phase body's surface slopes essentially downwards everywhere and that it is preferably provided at its lowest end with a sharp cusp (5/10).

8. Equipment as defined in claims 6 and 7, **characterized in that** the phase body has grooves or ridges (7) extending to the lowest end of the body.

9. Equipment as defined in anyone of claims 6 - 8, **characterized in that** it has an agitating means for agitating the medium in the vessel.

10. Equipment as defined in claim 9, **characterized in that** the phase body (4) functions as agitator.

11. Equipment as defined in anyone of claims 6 - 10, **characterized in that** the reaction vessel and at least one other vessel needed in the determination, preferably all vessels needed in the determination, are joined together to form one vessel unit (1).

under phase body arm 20, and the first step is carried out. The cassette is then moved step by step inwardly, until the last well is located at the measuring device.

Figure 4 also shows a diagrammatic view of energy-supplying unit 24, control unit 25, sample dosing pump 25, airing and diluter unit 26 and point washing well 27 in the equipment.

No fluid transfers need to be done in the determination, whereby it is possible to construct a safe, simple and reliably-operating system.

In the device according to Figure 2 there are a thin rod 3.1 and a solid-phase body 4.1 whose outer surface consists of two basically conical surfaces 8 and 9 curving inwardly. The rod diameter may be, for example, about one-tenth of the phase body diameter. Coating with the separating reagent should be done in such a way that also the rod is coated along its whole length coming into contact with the reaction solution. This is to prevent any unspecific reactions from taking place on the uncoated part. However, as the rod area is small compared to the phase body area, dosing exactitudes during the process will not have any strong effect on the treatment area of the coating.

The curved surfaces 8 and 9 of body 4.1 increase the surface area. The sharp point 10 and the sharp junction 11 of the surfaces make it easier for the fluid to flow off the body. The bottom 13 of vessel 12 is shaped to correspond with the body shape at its edges, but there is a level area in the middle.

Bodies consisting of several parts can be made for multi-runs with the different parts coated with different reagents. For example, the body shown in Figure 1 could consist of two vertical drop-halves, whereas the body shown in Figure 2 could consist of two conical parts.

Figure 3 shows a set of equipment where ten determinations can be performed at the same time.

Determination plates 1 are placed in a cassette 14. At the end of the last well in each plate there is a code 15 informing the equipment about the determination in question. The code can also give other information, especially the ageing time.

Cassette 14 is pushed into the equipment in the longitudinal direction of the plates with the code end first through opening 16, whereafter the cassette is moved automatically. The equipment has a detector end 17 movable in a transverse direct-ion and provided with an identifying device 18 for reading the code and with a measuring device 19 for finding

surface is preferably provided with a small gap between the vessels. This will reveal possible leaks under the film leading from one vessel to another.

The equipment may also have a safeguarding system which checks that there is medium in the vessel before the step is started. The phase body may function conveniently as the detector in such a system based on electrical conductivity measurement.

If desired, some suitable substance may be attached into that reaction vessel, into which the sample is brought, whereby the substance is attached to the vessel wall or to a separate solid phase remaining in the vessel. This substance will bind any such substances from the sample or from the formed complex that may disturbe later determination steps.

The plate vessels are preferably in a single straight row, whereby you need to move the phase body only along a straight path in a horizontal plane in relation to the plate. The vessels of different steps may be located in any order in relation to each other. The vessels are preferably permanently fixed to one another. The plate may be made of some suitable material, preferably of plastic.

The plate is preferably provided with detent pins and the equipment with their counterparts, so that the plate can not be placed in a wrong position by mistake.

In the following some embodiments of the invention are described by way of example. In the appended drawings

- Figure 1 shows implementation of the method by using a solid-phase body,
- Figure 2 shows an alternative solid-phase body,
- Figure 3 shows equipment suitable for use when carrying out the method, and
- Figure 4 shows another set of equipment of greater capacity.

According to Figure 1, immunodetermination is performed by using a plate 1, which consists of seven wells 2 located in a straight row, and a rod 3 having a solid-phase body 4 and a separating reagent (e.g. an antibody) reacting with the analyte (for example, an antigen) to be determined and attached to the body surface.

and the separating reaction is allowed to take place. Then intermediate steps, if such are required, are carried out in the other vessels and the phase body is finally moved over into the measuring vessel. Mediums needed in the determination are dosed beforehand into the vessels.

The phase body is of a cross-section similar to the reaction vessel, usually a circular one. In this way the diffusion distances from the solution to the solid phase will be as short as possible. In addition, the body can be used for efficient agitating of the reaction mixture.

The surface of the phase body is preferably of such a shape that fluid will flow off the surface as completely as possible. The best shape is oval with a tip at the bottom. However, the solid body may have, for example, several pins which are directed downwards. To enlarge the surface area, there may be suitable grooves or nodules on the body's surface. The reaction vessel bottom is preferably given the same shape as the phase body so that as little medium as possible will be required.

The vessels are preferably made as one unit. However, it is possible in principle to perform a part of the steps outside the vessel unit, especially the measurement of the formed reaction product, should this be desirable. Using an outside measuring vessel could be suitable especially if the complex is observed directly from the solid phase, for example, fluoro-metrically or radiometrically.

Correspondingly, several steps may be performed in the same vessel, for example, washes. Medium may also be dosed into some vessel or removed from it. Using separate dosings might be suitable in those steps where exact dosing is not needed and where, for example, the same medium is used in several differ-ent determinations. Washes, in particular, could be such steps. However, in normal cases it is most advantageous to use such vessel units where all different mediums are ready in different vessels.

Washes at least are usually performed in intermediate determination steps. In addition, in intermediate steps the formed reaction complex is usually joined to a tracer which is then detected in the measuring step. The tracer may be

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic	SD	Sudan
CG	Congo		of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				